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Phylogenetic diversity of the *Pristimantis anaiae* species group, with a description of a new species of *Pristimantis* (Anura, Strabomantidae) from Cerro Candelaria, Tungurahua, Ecuador

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Abstract

Continuous field work on mountains in the upper Pastaza watershed, showed an unexpected cryptic diversity in the recently described *Pristimantis anaiae* species group. Based on molecular and morphological characters we describe a new frog species from the central region of the eastern Ecuadorian Andes. We present an updated phylogeny for the *Pristimantis anaiae* species group, based on widely used, ubiquitous, and available genes, three mitochondrial genes (12S rRNA, 16S rRNA, ND1) and RAG1 nuclear gene. Our phylogenetic hypothesis increases genetic diversity of the species group and shows two well-supported subclades. We show the relationships of the new species and include for the first time *Pristimantis maryanneae*, belonging to a clade related to *P. resistencia* + *P. glendae*. Our phylogeny shows that new species, from Cerro Candelaria, is more closely related to *P. kunam* from Sangay National Park and differs from it by having a less conspicuous dorsolateral row of enlarged tubercles, and sacral oval marks surrounded by thin white pale lines. The new species and two additional undescribed species occur on the south side of the Rio Pastaza Valley, at Cerro Candelaria within the Llanganates – Sangay Ecological Corridor, reflecting the high importance of this area for conservation and understanding of evolution processes in the area, suggesting a probably adaptive radiation zone for the *P. anaia*e species group.

Key Words

Andes, amphibia, nanopore sequencing, upper Rio Pastaza watershed, *Pristimantis normaewingae* sp. nov.

Introduction

Pastaza River Valley is located in the eastern Cordillera in central Ecuador; this region is between the Andes and Amazon and in the middle of two National Parks, Llanganates and Sangay. As a whole, this region comprises a wide gradient of altitude, climate, and ecosystems (94,362 hectares, ranging from 760 meters to 3,812 meters in elevation); resulting in one of the most biodiverse and endemic places on Ecuador, with deep implications and high value for connectivity, biogeography, evolution and conservation, as seen in mammals, amphibians, birds and plants (Lynch and Duellman 1980; Gaglio et al. 2017; Freile et al. 2022; Clark et al. 2023; INABIO et al. 2023; Ríos-Alvear et al. 2024).

Pristimantis is a species-rich genus of frogs in the northern Andes of South America, and its known richness has increased exponentially in the last decade (Reyes-

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Puig and Mancero 2022). The number of endemic and threatened species in the genus in the tropical Andes of Ecuador is growing rapidly; 14 new species of this genus have been described in the upper Rio Pastaza watershed since 2010, most of these species being endemic to the region (Reyes-Puig et al. 2022; INABIO et al. 2023; Reyes-Puig et al. 2023).

Within the Llanganates – Sangay Corridor (CLS), different types of conservation areas have been created. Cerro Candelaria Private Protected Area is one of the biggest conservation efforts in the CLS, protecting 2696 hectares along a wide gradient (1500 to 3840 m), including ecosystems from cloud forest to paramo. This area is the type locality for five new species of frogs to date (INABIO et al. 2023). Despite numerous extensive research expeditions within the Cerro Candelaria Private Reserve and the northernmost part of Sangay National Park, some areas remain unexplored, with undiscovered biodiversity. Recent expeditions have revealed novel species, especially in the particularly difficult genus *Pristimantis* (Brito and Almendariz 2013; Yanez-Muñoz et al. 2013; Ecominga and INABIO 2022).

A recent publication revealed cryptic diversity in an unknown, strongly supported clade of *Pristimantis* thanks to availability of genetic information; it was named the *Pristimantis anaiae* species group (Ortega et al. 2022). This group is characterized by lacking vocal slits and tympanic membrane and by having large dark round areas with thin clear borders in the sacral region (Ortega et al. 2022). In the last few years, new expeditions to the mountains south of the Rio Pastaza revealed additional new specimens related to this *Pristimantis anaiae* species group, and one of them is described herein.

Materials and methods

Nomenclature and morphological analysis

For new species description and diagnosis, we followed standard terms used by Lynch and Duellman (1997), with adaptations to the *Pristimantis anaiae* species group (Ortega et al. 2022). We followed the diagnostic characters, definitions, and diagrams applied by Duellman and Lehr (2009). Also, criteria proposed by Heinicke et al. (2007), Hedges et al. (2008), and Padial et al. (2013) for family systematic classification were used.

Specimens were collected during field expeditions and herpetology inventories, whose techniques are detailed in Yánez et al. (2013) and Ecominga and INABIO (2022). We euthanized specimens using benzocaine and collected specimens were fixed on 10% formalin for twenty-four hours and later preserved in 70% ethanol. Sex and maturity were determined by secondary sexual traits identification (vocal slits and nuptial pads in males, body size), and direct gonad inspection through dorsolateral incisions.

Morphometric measures were taken with an electronic caliper (precision ± 0.01 mm), following Duellman and Lehr (2009): SVL (snout–vent length), TL (tibia length), FL (foot length, distance from proximal margin of inner metatarsal tubercle to tip of Toe IV), HL (head length, obliquely from the angle of the jaw to tip of snout), HW (head width, at the level of angle of jaw), ED (eye diameter), IOD (interorbital distance), EW (upper eyelid width), IND (internarial distance), E-N (eye–nostril distance, straight line distance between anterior corner of orbit and posterior margin of external nostrils). Fingers were numbered preaxially to post axially from I–IV. Comparative lengths of Toes III and V were determined when adpressed against Toe IV; lengths of Fingers I and II were estimated when adpressed against each other.

The coordinates and elevation of type localities were determined based on GPS using Datum WGS 84 records and collector field notes. The following abbreviations were used for photograph recognition and related information: Juan P. Reyes-Puig (JPRP), Julio C. Carrión-Olmedo (JCC), Mario H. Yanez Muñoz (MYM), Patricio Vinueza (PV), Zane Libke (ZL), and Bioweb Santiago R. Ron (BioWeb SRR).

Type specimens and additional specimens examined (Appendix 1) were deposited in the herpetology repository of the Ecuadorian Museum of Natural History (DHMECN). Tissue samples and genetic material were stored at -80° Celsius in the Nucleic Acid Sequencing Laboratory of INABIO.

DNA extraction, amplification, sequencing, and bioinformatics

DNA extraction, PCR amplification, and subsequent nanopore sequencing were performed at the Nucleic Acid Sequencing Laboratory of the Instituto Nacional de Biodiversidad (INABIO) in Quito and Sumak Kawsay in Situ (SKIS) in the Pastaza rainforest.

DNA was extracted from the liver or muscle under standard protocols using GeneJET Genomic DNA Purification Kit (K0722) or Omega Biotek E.Z.N.A Tissue DNA Kit (D3396). Gene amplification was performed by Polymerase Chain Reaction (PCR) for four different DNA fragments, three contiguous mitochondrial genes 12S rRNA, 16S rRNA, and NADH dehydrogenase subunit 1 (ND1), and one nuclear gene Recombination Activating Gene 1 (RAG1). 12.5uL reactions consisted of 6.25uL 2X Dreamtaq Hotstart Mastermix (ThermoFisher), 2uL DNA template, 1uL Forward Primer, 1uL Reverse primer, and 2.25uL nuclease-free water.

We amplified the Recombination Activating Gene 1 (RAG1) using R270 and R182 primers and standard protocols (Hedges et al. 2008). Mitochondrial genes were amplified in continuous fragments using a combination of primers flanking more than one gene to exploit the advantages of long-read sequencing. We targeted a 2000bp fragment of 12S rRNA and a partial fragment of 16S rRNA using 12sL4E and 16H36E (Heinicke et al. 2007), and a 2500bp complementary and overlapping fragment of 16S rRNA with NADH dehydrogenase subunit 1 gene

(ND1) using 16L19 and t-Met-frog (Wiens et al. 2005; Moen and Wiens 2009).

Chosen molecular markers (12S rRNA, 16S rRNA, ND1, and RAG1) are widely used in amphibian phylogenetics (Hay et al. 1995; Chan et al. 2022; Portik et al. 2023), are currently available for the genus *Pristimantis* and were used for the phylogenetic analysis by Ortega et al. (2022).

Three sequencing runs were performed at two different sites. At INABIO, sequencing was performed on a minION mk1c using Flongle Flow Cells R9.4.1 and Rapid Barcoding Kit 96 (SQK-RBK110.96) following standard protocols. At SKIS, sequencing was performed on a minION mk1b with Flongle Flow Cells R10.4.1, SQK-LSK114, and custom-designed indices (barcode generator). SKIS data was Super-accuracy (SUP) basecalled on Dorado 0.5.1 (model dna_r10.4.1_e8.2_400bps_sup@v4.3.0) and demultiplexed with minibar. INABIO data was High-accuracy (HAC) basecalled and demultiplexed with guppy 6.4.6.

Demuxed fastqs were filtered at a Qscore of 9, and consensus sequences were generated with NGSpeciesID (Sahlin et al. 2021). Despite the slight differences in the production of data between the two sites, all consensus sequences produced had between 100–1000 supporting reads, with an expected divergence from Sanger between 0.00–0.04% (Vasiljevic et al. 2021), too small to be consequential to robust phylogenetic analysis.

Phylogenetic analysis

A total of 86 consensus sequences were generated (25 of 12S, 32 of 16S, 16 of ND1, 13 of RAG1) of 32 individuals. Those sequences were imported and interpreted using Mesquite (Maddison and Maddison 2018). The newly generated sequences are available in GenBank (Table 1) as Chakrabarty et al. (2013) suggested. A BLASTn search was performed to include in the character matrix the most closely related lineages.

Character matrices were built independently for each fragment. Each matrix was aligned using default parameters in Muscle (Edgar 2004) and corrected visually for unambiguous alignment errors. The aligned matrices were concatenated and partitioned by gene, and in coding genes, by codon.

Substitution models and maximum likelihood tree inference were performed using IQ-TREE (Trifinopoulos et al. 2016) under default settings. Branch support was evaluated using 2000 ultrafast bootstrapping and SH-aLRT tests with 1000 replicates (Guindon et al. 2010). Phylogeny inference parameters and chosen molecular markers followed methodology used in Ortega et al. (2022). Uncorrected *p*-distances were calculated with an 800 bp long fragment of 16S rRNA using MEGA 11 (Tamura et al. 2021).

Results

Phylogenetic relationships and genetic distances (Fig. 1)

We included 32 new samples into the *Pristimantis anaiae* species group phylogeny presented by Ortega et al. (2022) as they are similar in 16S BLASTn procedure, resulted in 90%–95% within-group identity of the species.

The concatenated matrix was 3864 bp long for 46 individuals (available at https://doi.org/10.5281/zenodo.10463523). IQTree evaluated the best substitution

Table 1. GenBank Accession numbers for DNA sequences used for phylogenetic analyses.

Species	Citation	Type of type	Specimen Catalog	GenBank				GenSeq Nomenclature
				12S	16S	ND1	RAG1	
Pristimantis anaiae	Ortega et al. 2022	Holotype	QCAZ 59693	MZ330740	MZ241532	MZ18944	MZ332966	genseq-1
		Paratype	QCAZ 59720	NA	MZ241533	NA	MZ332964	genseq-2
		Paratype	QCAZ 59627	NA	MZ241525	MZ18944	MZ332957	genseq-2
		Paratype	QCAZ 59640	NA	MZ241527	MZ18944	MZ332959	genseq-2
Pristimantis glendae	Ortega et al. 2022	Holotype	QCAZ 56437	NA	MZ241511	NA	MZ332946	genseq-1
		Paratype	QCAZ45832	MZ330731	MZ241501	NA	MZ332937	genseq-2
		Paratype	QCAZ45784	NA	MZ241499	NA	MZ332935	genseq-2
		Paratype	QCAZ45793	NA	MZ241500	NA	MZ332936	genseq-2
		Non-Type	ZL131	PP102403	PP102437	PP101682	PP101666	genseq-3
Pristimantis maryanneae	This paper	Holotype	DHMECN14454	NA	PP102406	PP101667	NA	genseq-1
		Paratype	DHMECN18213	NA	PP102408	PP101669	PP101655	genseq-2
		Paratype	DHMECN18221	PP102381	PP102409	PP101670	NA	genseq-2
		Paratype	DHMECN18222	PP102382	PP102410	PP101671	PP101656	genseq-2
Pristimantis kunam	Ortega et al. 2022	Holotype	QCAZ 56438	NA	MZ241512	NA	MZ332947	genseq-1

Species	Citation	Type of type	Specimen Catalog	GenBank				GenSeq Nomenclature
				12S	16S	ND1	RAG1	
Pristimantis normaewingae sp. nov.	This paper	Holotype	DHMECN18409	PP102395	PP102423	NA	NA	genseq-1
		Paratype	DHMECN17191	PP102383	PP102407	PP101668	PP101654	genseq-2
		Paratype	DHMECN18391	PP102384	PP102411	NA	NA	genseq-2
		Paratype	DHMECN18393	PP102385	PP102412	NA	NA	genseq-2
		Paratype	DHMECN18397	PP102386	PP102414	NA	NA	genseq-2
		Paratype	DHMECN18398	PP102387	PP102415	NA	NA	genseq-2
		Paratype	DHMECN18400	PP102388	PP102416	PP101672	PP101657	genseq-2
		Paratype	DHMECN18402	PP102389	PP102417	NA	NA	genseq-2
		Paratype	DHMECN18403	PP102390	PP102418	NA	NA	genseq-2
		Paratype	DHMECN18404	PP102391	PP102419	NA	NA	genseq-2
		Paratype	DHMECN18406	PP102392	PP102420	NA	NA	genseq-2
		Paratype	DHMECN18407	PP102393	PP102421	NA	NA	genseq-2
		Paratype	DHMECN18408	PP102394	PP102422	NA	NA	genseq-2
		Paratype	DHMECN18410	PP102396	PP102424	NA	NA	genseq-2
		Paratype	DHMECN18411	PP102397	PP102425	NA	NA	genseq-2
		Paratype	DHMECN18412	PP102398	PP102426	NA	NA	genseq-2
		Paratype	DHMECN18861	NA	PP102427	PP101673	PP101658	genseq-2
		Paratype	DHMECN18862	PP102399	PP102428	PP101674	PP101659	genseq-2
		Paratype	DHMECN18863	PP102400	PP102429	PP101675	PP101660	genseq-2
		Paratype	DHMECN18864	NA	PP102430	PP101676	PP101661	genseq-2
		Paratype	DHMECN18865	PP102401	PP102431	PP101677	PP101662	genseq-2
		Paratype	DHMECN18866	NA	PP102432	PP101678	PP101663	genseq-2
		Paratype	DHMECN18868	PP102402	PP102434	PP101679	PP101664	genseq-2
		Paratype	DHMECN18869	NA	PP102435	PP101680	NA	genseq-2
		Paratype	DHMECN18996	NA	PP102436	PP101681	PP101665	genseq-2
Pristimantis resistencia	Ortega et al. 2022	Holotype	QCAZ66519	OM730030	OM729993	NA	NA	genseq-1
		Paratype	QCAZ66523	OM730032	OM729995	NA	NA	genseq-2
		Paratype	QCAZ66467	OM730031	OM729994	NA	NA	genseq-2
Pristimantis sp. 1	This paper	Non-Type	DHMECN18867	PP102404	PP102433	NA	NA	genseq-3
Pristimantis sp. 2	This paper	Non-Type	DHMECN18395	PP102405	PP102413	NA	NA	genseq-3
Pristimantis venegasi	Ortega et al. 2022	Holotype	QCAZ66440	OM730029	OM729992	NA	OM752308	genseq-1
		Paratype	QCAZ31130	NA	MZ241484	NA	MZ332921	genseq-2

model for 8 partitions as follows: TIM2+F+G4 for 12S rRNA, TIM2+F+I+G4 for 16S rRNA, TPM2u+F+I+G4 for ND1 first position, TIM2e+G4 for ND1 second position, TN+F+G4 for ND1 third position, K2P+G4 for RAG1 first position, K2P+G4 for RAG1 second position, and K3P+G4 for RAG1 third position.

Our data is congruent with Ortega et al. (2022) and confirms the monophyly of the *Pristimantis anaiae* species group, maintaining its strong support (100/100). However, new topology within the *Pristimantis anaiae* species group showed inconsistencies with previous inferences. Differences between our phylogeny and the previous one for the *Pristimantis anaiae* species group (Ortega et al. 2022) are due to new subclades A and B (Fig. 1) formed by including new lineages, one of them described as a new species herein.

The *Pristimantis anaiae* species group is divided into two clades. Subclade A is composed of two species, *P. kunam* and its sister species, *P. normawingae* sp. nov. (86.5/99 branch support). Subclade B is formed by two branches, one branch (85.8/99 branch support) formed by *P. venegasi* and a sister clade (*P. anaiae* + *P.* sp. 1), and the second branch (100/98) is composed by *P. glendae* as the sister species of *P. resistencia* (82/91 branch support), and both formed the sister clade of *P.* sp. 2 (100/95 branch support). This new clade *P.* sp. 2 + (*P. resistencia* + *P. glendae*) is the sister clade of *P. maryanneae* (100/98 branch support).

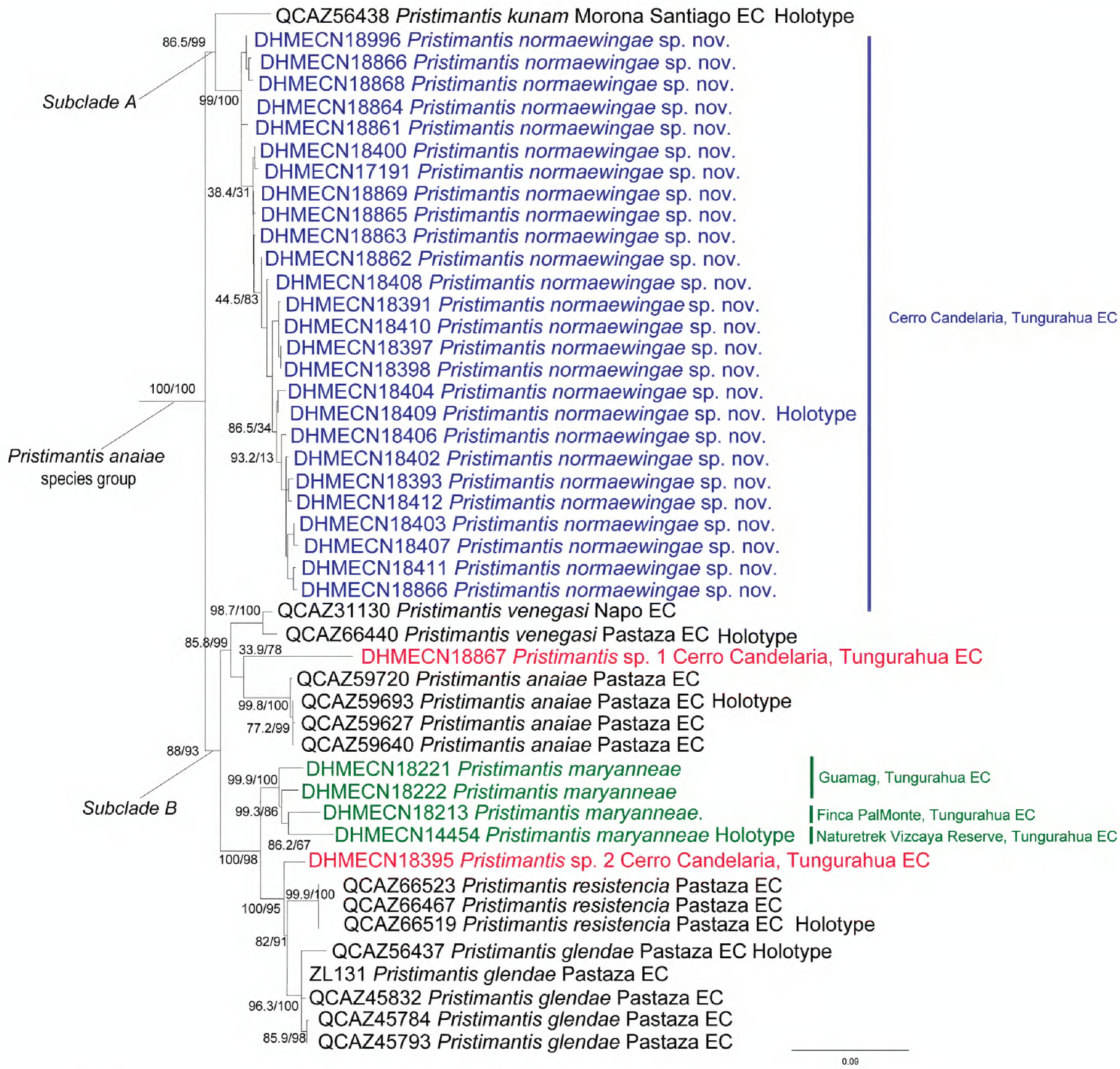

Figure 1. Phylogenetic relationships among the *Pristimantis anaiae* species group, with the inclusion of *Pristimantis normaewingae* sp. nov., *Pristimantis maryanneae*, and two candidate species (*Pristimantis* sp. 1, *P.* sp. 2).

Table 2. Genetic p-distances of the *Pristimantis anaiae* species group. Average (minimum – maximum).

	P. kunam	*P. normaewingae* sp. nov.	*P. venegasi*	*P. anaiae*	*P. maryanneae*	*P. esistencia*	*P. glendae*
Pristimantis kunam		(5.15%–7.55%)	(8.47%–11.82%)	(8.49%–8.65%)	(9.71%–18.71%)	(10.05%–10.97%)	(8.54%–8.76%)
***Pristimantis normaewingae* sp. nov.**	5.93%		(5.23%–12.45%)	(6.57%–8.94%)	(6.64%–14.68%)	(8.72%–13.76%)	(6.67%–9.42%)
Pristimantis venegasi	10.15%	7.29%		(6.28%–7.06%)	(6.28%–16.94%)	(5.84%–6.40%)	(6.20%–10.47%)
Pristimantis anaiae	8.59%	7.96%	6.64%		(7.04%–15.83%)	(3.02%–3.87%)	(7.05%–7.39%)
Pristimantis maryanneae	12.21%	9.34%	10.42%	9.75%		(7.76%–15.34%)	(5.14%–14.98%)
Pristimantis esistencia	10.52%	9.70%	6.11%	3.58%	11.42%		(9.64%–11.42%)
Pristimantis glendae	8.65%	7.50%	8.39%	7.20%	8.12%	10.31%	

The 16s rRNA uncorrected *p*-distance between *Pristimantis kunam* and *P. normawingae* sp. nov. averages 5.93% (5.15%–7.55%), and the uncorrected *p*-distance between *P. glendae* and *P. maryanneae* averages 8.12% (5.13–14.97 %). Genetic *p*-distances are shown in Table 2.

Systematics accounts

Pristimantis normaewingae sp. nov.

https://zoobank.org/A8472152-7B5F-4D2A-9C91-24B65265C2FA

Figs 1–3

English Common name: Norma Ewing Rain Frog.

Spanish Common name: Cutín de Norma Ewing.

Holotype. Adult female. Ecuador; Tungurahua Province, Cerro Candelaria Private Protected Area; 1°27.410'S, 78°18.192'W, 3042 m a.s.l.; collected by JPRP, PV, Darwin Recalde, Eduardo Peña, Paulette Benavides and Nantar Kuja on 23 November 2022; on a shrub branch covered with moss at 170 cm above the ground; GenBank: PP102395, PP102423 ; DHMECN 18409.

Paratypes. Ecuador • (6 ♀, 23 ♂, 9 juveniles); four adult females (DHMECN 18861, DHMECN 18863, DHMECN 18865, DHMECN 18868); from the same protected area near holotype locality; 1°26.301'S, 78°18.105'W; alt. 2626 m; collected by JPRP, Evelyn Toapanta, Elizabeth Chicaiza Toa and Luis Recalde on 22–23 February 2023; Two adult females (DHMECN 4849, and DHMECN 4846); from the same locality as the holotype; 1°26.399'S, 78°17.991'W; alt. 2700 m; 5 May 2008; collected by JPRP and Luis Recalde.

Thirteen adult males (DHMECN 18392, DHMECN 18396, DHMECN18393, DHMECN 18397, DHMECN 18401, DHMECN 18402, DHMECN 18403, DHMECN 18404, DHMECN 18406, DHMECN 18407, DHMECN 18411, DHMECN 18412, DHMECN 18414), collected in the same locality and date as the holotype. Two adult males (DHMECN 18862, DHMECN 18896); collected in the same locality and date as DHMECN 18861. Four adult males (DHMECN 17188, DHMECN 17189, DHMECN 17191, DHMECN 17192); collected in the same locality as the holotype by JPRP and ZL on 18 January 2021. Two Adult males (DHMECN 4847, DHMECN 4975); collected in the same locality and data as DHMECN 4849.

Female juvenile (DHMECN 17190), collected in the same locality and data as DHMECN 17188. Five male juveniles (DHMECN 18398, DHMECN 18400, DHMECN 18405, DHMECN 18408, DHMECN 18413); collected in the same locality and data as the holotype. Male juvenile (DHMECN 18864); collected in the same locality and data as DHMECN 18861.

Diagnosis. We assign the new species to the genus *Pristimantis* and the *Pristimantis anaiae* species group based on molecular phylogeny (Fig. 1). *Pristimantis normaewingae* sp. nov. is characterized by the following combination of characters: (1) skin of dorsum shagreen with small low tubercles, skin on throat areolate, skin on belly strongly areolate bearing scattered tubercles; (2) discoidal and thoracic fold present; (3) dorsolateral folds fragmented, instead a dorsolateral row of enlarged tubercles is present in the posterior back, more evident in males; (4) tympanic membrane absent, tympanic annulus present small (on average, 40.99% of eye diameter); (5) supratympanic fold present with low tubercles, a pair of prootic and occipital tubercles aligned with several subconical tubercles aligned forming a "W" in the scapular region; (6) several subconical and rounded postrictal tubercle present; (7) snout short and subacuminate in dorsal and rounded in lateral view, with 4 tubercles aligned with canthal region forming a cross in dorsal view; (8) upper eyelid with one conical tubercle and few lower tubercles; (9) cranial crests absent; (10) Dentigerous processes of vomers present, oblique, separated; vocal slits and nuptial pads absent; (11) Finger I shorter than Finger II, discs of digits expanded to broadly expanded, truncate; (12) fingers with narrow lateral fringes, all fingers with elongated and thin hyperdistal tubercles; (13) low and rounded ulnar tubercles, more conspicuous in males, low ulnar fold evident ventrally; (14) heel bearing a low subconical or rounded tubercle; row of subconical or rounded tubercles along external tarsus; (15) toes with broad lateral fringes, basal webbing absent, all toes with elongated hyperdistal tubercles; (16) Toe V much longer than Toe III, Toe condition (Toe III surpasses the distal border of the distal subarticular tubercle of Toe V and reaches the distal border of the penultimate tubercle of Toe IV; Toe V reaches towards the outermost border of the distal tubercle of toe IV); (17) In life, dorsal coloration is composed by irregular marks mixed with banded pattern with different tones of brown, yellow and green; dark canthal, subocular and supratympanic stripes present, middorsal oval or irregular dark marks delineated by pale lines, surrounded by irregular contrasting marks, flanks with brown or greenish-brown tones, dark incomplete oval marks in sacral region with thin light borders are present on each side. Ventral surfaces of the belly, throat, and limbs are marmorated with dark brown and cream or gray marbling. In males, light reddish or pink flecks are slightly evident in palms and soles, and part of ventral portions of limbs, while in females reddish or pink tones are absent in such ventral surfaces; the iris is light blue with a horizontal wide copper medial band and black reticulations; light pale sclera; (18) SVL in females, 25.0–30.6 mm (mean = 28.14, SD = 1.94, n = 7); SVL in adult males, 16.7–22.27 mm (mean = 19.68, SD = 2.49, n = 20); Tables 3, 4.

Comparison with other species. (Fig. 4, Table 4) *Pristimantis normaewingae* sp. nov. can be distinguished from all seven species of the *Pristimantis anaiae* species group by having incomplete oval marks in sacral region, a row of enlarged dorsolateral tubercles and a light blue iris with a copper horizontal wide medial band with black reticulations; while the other species present different iris patterns, complete oval marks in sacral region and lacks dorsolateral row of enlarged tubercles like *P. anaiae*, *P. resistencia*, *P. glendae* and *P. venegasi*, with the excep-

Table 3. Measurements of *Pristimantis normaewingae* sp. nov. and *P. maryanneae* in mm, with means and standard deviations in parentheses. See materials and methods for abbreviations, based on Duellman and Lehr (2009).

	Pristimantis normaewingae **sp. nov.**		*Pristimantis maryanneae*	
	Females n = 7	**Males n = 20**	**Females n = 3**	**Males n = 13**
SVL	25.04–30.56 (28.14 ± 1.94)	16.7–22.27 (19.68 ± 1.58)	21.10–25.30 (23.60 ± 2.21)	17.80–20.94 (19.46 ± 0.87)
HW	10.40–11.54 (11.13 ± 0,51)	6.66–8.55 (7.47 ± 0.52)	8.90–9.94 (9.59 ±0.69)	6.08–8.35 (7.49 ± 0.55)
HL	9.77–11.59 (11.03 ± 0.59)	7.1–9.07 (8.13 ± 0,57)	8.00–10.30 (9.42 ± 1.24)	7.10–8.37 (7.81 ± 0.41)
EN	2.41–2.99 (2.77 ± 0.22)	1.57–2.19 (1.97 ± 0.21)	2.10–2.52 (2.32 ± 0.21)	1.76–2.15 (1.91 ± 0.12)
IND	2.00–2.63 (2.24 ± 0.20)	1.57–1.98 (1.78 ± 0.11)	2.00–3.40 (2.57 ± 0.73)	1.82–1.27 (1.85 ± 0.13)
IOD	3.31–3.85 (3.55 ± 0.38)	2.05–2.98 (2.55 ± 0.21)	2.40–3.22 (2.94 ± 0.47)	2.31–2.98 (2.60 ± 0.20)
EW	2.07–2.86 (2.56 ± 0.26)	1.19–2.39 (1.94 ± 1.99)	1.50–2.68 (2.22 ± 0.63)	1.70–2.30 (2.04 ± 0.22)
TD	0.93–1.93 (1.32 ± 0.28)	0.57–1.16 (0.86 ± 0.17)	1.00–1.08 (1.00 ± 0.09)	0.50–0.96 (0.77 ± 0.12)
ED	3.02–3.69 (3.22 ± 0.22)	1.88–2.75 (2.44 ± 0.24)	2.70–3.10 (2.80 ± 0.26)	2.30–2.74 (2.49 ± 0.14)
TL	12.70–15.93 (14.82 ± 1.10)	9.15–12.28 (10.58 ± 0.91)	10.30–13.38 (12.13 ± 1.62)	8.90–12.06 (10.32 ± 0.75)
HaL	7.54–9.20 (9.22 ± 0.86)	5.72–6.82 (6.17 ± 0.56)	7.90–7.77 (5.22 ± 3.69)	5.81–6.67 (6.15 ± 0.27)
FL	12.27–15.65 (14.26 ± 1.03)	4.72–7.58 (6.15 ± 1.07)	9.40–12.42 (11.31 ± 1.66)	8.40–10.06 (9.40 ± 0.52)
FW	1.44–1.88 (1.71 ± 0.16)	0.87–1.39 (1.20 ± 0.17)	1.40–1.59 (1.00 ± 0.72)	0.97–1.32 (1.16 ± 0.08)
TW	1.75–1.27 (1.60 ± 0.16)	0.3–0.6 (1.16 ± 0.17)	1.17–1.30 (0.82 ± 1.73)	0.91–1.31 (1.15 ± 0.11)

tion of *P. kunam* and *P. maryanneae*, that respectively present larger and smaller dorsolateral row of enlarged tubercles. *P. kunam* is the smallest species of the group (SVL: 14.66), however, *Pristimantis normaewingae* sp. nov. interspecific SVL variation overlaps with the other species of the group, and has less conspicuous ulnar tubercles and light brown venter in comparison with the conspicuous conical ulnar, and tarsal tubercles, dark greenish with dark flecks body coloration and bronze iris with black reticulations of *P. kunam*. *P. maryanneae* present less tuberculated skin, tympanum absent, small and inconspicuous row of dorsolateral tubercles, and pale ventral surfaces than *P. normaewingae* sp. nov. that has contrasting body and iris coloration, tympanum hidden beneath the skin with evident tympanic anulus and more tuberculated skin. Detailed comparisons of diagnostic characters between all species of *Pristimantis anaiae* group are shown in Table 4.

Description of the holotype. (Figs 2, 3) Adult female (DHMECN 18409). Head wider than long, slightly wider than the body, snout acuminate in dorsal view, short and rounded in profile; canthus rostralis slightly rounded in dorsal view, concave in lateral view, four rounded tubercles aligned in a cross pattern in dorsal view; loreal region slightly concave; cranial crests absent, upper eyelid with a subconical tubercle surrounded by few smaller tubercles, interorbital tubercle present; tympanic annulus oval distinct beneath the skin; tympanic membrane absent; several rounded low postrictal tubercles and irregular warts; dark supratympanic fold present, distinct, covered with rounded subconical tubercles.

Dentigerous processes of vomers present, oblique, separated, posteromedial to choanae; each vomer bearing several inconspicuous small teeth. Skin on dorsum shagreen with small low tubercles, skin on flanks shagreen with small low tubercles, dorsolateral folds fragmented, instead a dorsolateral rows of rounded low tubercles are present more evident in the posterior back, discoidal and thoracic fold present; skin on throat coarsely areolate with some scattered low warts, chest and belly areolate with some low rounded tubercles; ventral surfaces of thighs areolate; skin in upper cloacal region strongly areolate; row of low subconical ulnar tubercles present, a low ulnar fold is evident ventrally; palmar tubercles prominent and elevated, outer palmar tubercle large bifid "horseshoe" shape well differentiated, thenar tubercle is oval and half size than the outer; subarticular tubercles well-defined, pronounced round in ventral and lateral view; hyperdistal tubercles flattened in ventral and lateral view; indistinct supernumerary tubercles; lateral fringes on fingers wide; Finger I shorter than Finger II; disc on Finger I slightly expanded and oval, discs on Fingers, II, III and IV widely expanded truncate; pads on all fingers well defined and surrounded by circumferential grooves (Fig. 3).

Table 4. Diagnostic morphological characters of the *Pristimantis anaiae* species group and its seven species.

Species	Iris Color	Dorsal Color	Ventral Color	Groins And Shanks	Digital Pads Shape	Tympanum And Tympanic Annulus Condition
Pristimantis normaewingeae sp. nov.	Light blue with horizontal wide coppery medial band and black reticulations.	Banded patterns in different brown, green, black, and reddish tones in some individuals, incomplete rounded areas on the flanks bordered by thin white borders.	Ventral surfaces heavily pigmented with brown marbling, in life males present reddish colorations on ventral surfaces of feet and hands.	Bands on dorsal limbs and flanks, incomplete rounded areas on the flanks bordered by thin white borders.	Large oval.	Tympanic annulus present, tympanum absent.
Pristimantis maryanneae	Gray with thin black reticulations and coppery horizontal stripe.	Banded and striped morphos in different brown tones.	Light ventral surfaces with minute dark flecks and thin dark line along the midbody.	Thighs and groins in several tones of brown and sometimes with faint light pink and yellow tones.	Large oval.	Tympanic annulus present, tympanum present.
Pristimantis kunam	Dark bronze iris with fine black reticulation.	Dorsum dark brown with a row of large orange conical tubercles extending along the dorsolateral line.	Ventral surfaces dark greenish with dark flecks.	Large sacral dark round areas with thin clear borders.	Small oval.	Tympanic annulus present, tympanum absent.
Pristimantis anaiae	Iris bronze with a wide coppery medial band and black reticulations; light blue sclera.	Dorsum greenish brown with an H-shaped greenish-orange mark, a large sacral dark round area with thin clear borders is present on each side.	Ventral surfaces cream to dark brown bearing black or dark brown tubercles.	Flanks pale orange to greenish brown bearing a short brown longitudinal stripe or a dark brown, circular blotch on its posterior portion.	Expanded truncate.	Tympanic membrane absent, tympanic annulus small.
Pristimantis resistencia	Coppery iris with thick black reticulation.	Dorsum light brown, dark reddish brown or dark greenish brown with two dorsal triangles.	Ventral surfaces brownish cream with dark brown patches.	inguinal and sacral markings of dark brown color and pale edges.	Truncate to blunt pads.	Tympanum and tympanic annulus present.
Pristimantis glendae	Iris greenish white to white with dark reticulations.	Dorsum orange, yellow or yellowish brown with scapular blotches warm sepia with dark bars with thin light lines.	Belly whitish cream full of small black flecks or mottled with a medial black line.	belly whitish cream full of small black flecks or mottled with a medial black line.	Slightly expanded.	Tympanum absent and tympanic annulus present.
Pristimantis venegasi	Bronze colored iris with a transverse coppery stripe and thick black reticulations.	Brown-green blotches with cream edges; large sacral dark round areas with thin clear cream-colored edges on sacral areas.	Black and white marbling.	Large sacral dark round areas with thin clear brown-green blotches with cream edges; large sacral dark round areas with thin clear lines.	Expanded truncate.	Tympanum and tympanic annulus present.
Pristimantis maryanneae	Light brown to gray with black reticulation and horizontal coppery stripe.	Dark gray to gray with green marks, with transverse dark brown marks, and a chevron and irregular "H" shaped marks on scapular region.	Dirty cream with a line along middle of the venter, chin and outer mandibula mottled with dark brown marks.	Flanks and shanks with cream diagonal bands, and light brown interspaces, hind-limbs with grey transverse bands and dark brown interspaces.	Expanded truncate.	Tympanum absent, hidden beneath the skin; tympanic annulus visible under the skin measuring.

Hindlimbs slender; upper surfaces of hindlimbs with scattered low tubercles; posterior and ventral surfaces of thighs rugose; heel bearing low subconical tubercle and smaller surrounding; outer surface of tarsus present a row of low subconical tubercles; tarsal fold not evident; well-defined inner metatarsal tubercle, large oval, elevated in lateral view, outer metatarsal prominent rounded about third size of the inner; plantar surface with subarticular tubercles rounded and well defined, subarticular tubercles well-defined, slightly prominent and rounded; hyperdistal tubercles flattened in ventral and lateral view; toes with wide lateral fringes, widened on Toes IV – III – II – I; basal webbing between toes absent; discs nearly as large as those on fingers, expanded in all toes specially

Figure 2. Preserved holotype (DHMECN18409, adult female) of *Pristimantis normaewingae* sp. nov, in dorsal, ventral, and lateral views. Photographs JPRP.

Figure 3. Ventral views of hand (left) and foot (right) of female holotype (DHMECN 18409) of *Pristimantis normaewingae* sp. nov. Photographs JPRP.

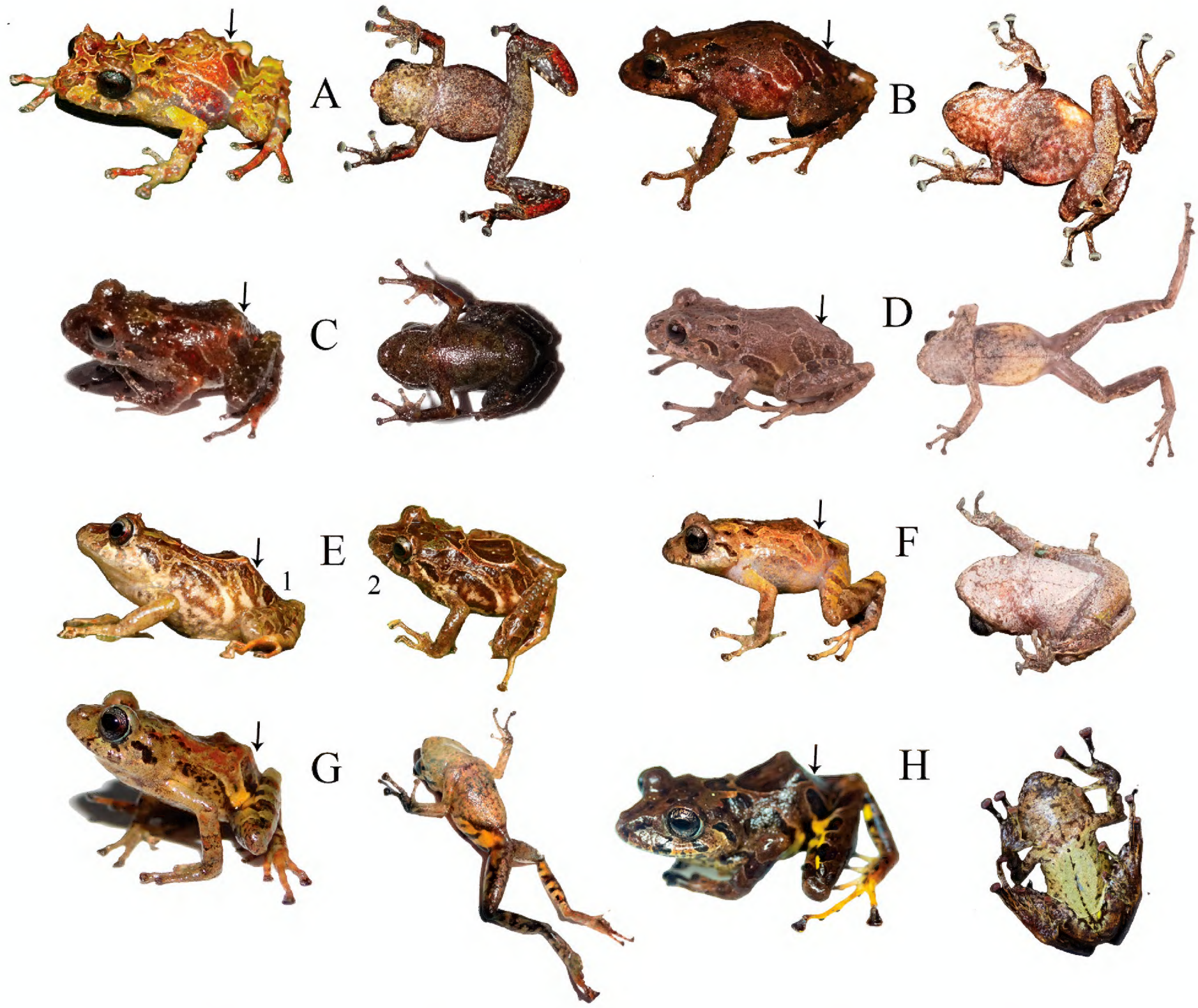

Figure 4. Life comparison between members of *Pristimantis anaiae* species group. **A.** *P. normaewingae* sp. nov. (DHMECN 18400, male paratype); **B.** *P. normaewingae* sp. nov. (DHMECN 18409, female holotype); **C.** *P. kunam* (QCAZ 56438, male holotype,); **D.** *P. resistencia* (QCAZ 66519, female holotype); **E.** *P. anaiae* (DHMECN 16859, male); **F.** *P. maryanneae* (DHMECN 18227, male); **G.** *P. glendae* (QCAZ 56437, adult male); **H.** *P. venegasi*, (MZUTI6571, female). Arrows show area of the oval marks in sacrum, proposed diagnostic of the group by Ortega et al. 2022. Photographs JPRP, MYM, JCC, Jorge Brito, and Bioweb.

on Toes III, IV and V, Toe I oval; all discs have pads surrounded by well-defined circumferential grooves; relative lengths of toes I < II < III < V < IV; Toe V much longer than Toe III (Toe III reach the hyper distal tubercles of Toe V and reaches the distal border of the medial subarticular tubercle of Toe IV; Toe V reaches the proximal border of the distal tubercle of Toe IV) (Fig. 3)

Measurements of the holotype (in mm). SVL = 30.56; Tibia Length = 15.93; Foot Length = 14.91; Hand Length = 9.82; Head Length = 11.36; Head Width = 11.15; Eye Diameter = 3.69; Tympanum Diameter = 1.79; Forearm Length = 7.09; Snout Length = 4.75; Tarsus Length = 8.59; Thigh Length = 12.72; Upper Arm Length =5.86; Interorbital Distance = 3.85; upper Eyelid Width = 2.59; Internarial Distance = 2.0; Eye–Nostril distance = 2.88; Finger III width = 1.88; Toe IV Width = 1.75.

Color of holotype in life. (Fig. 5) Dorsal coloration dark brown with diagonal bands on flanks and limbs, anterior snout light brown, dark subocular and supratympanic marks, W-shaped dark blotch present on scapular region, underlined by white lines. Flanks with diagonal dark brown bands, surrounded by pale lines, hidden surfaces of the groin and thighs brown. Tip of the digits with pale color. All ventral surfaces are light brown marbled or marmorated with darker brown tones. Iris coloration pale gray with light blue at peripherical surfaces, extensive fine dark reticulations and medial horizontal copper stripe is present, white sclera.

Color of holotype in preservative. (Fig. 6) Dorsal coloration of body and limbs composed of a dark brown with diagonal dark bands delineated with thin white lines, incomplete oval sacral marks delineated by white on the back, bold dark scapular chevron delineated by pale line, tips of the digits with pale marks. Ventral surfaces are light brown and marmorated with dark brown. Pale disc surfaces on hands and feet.

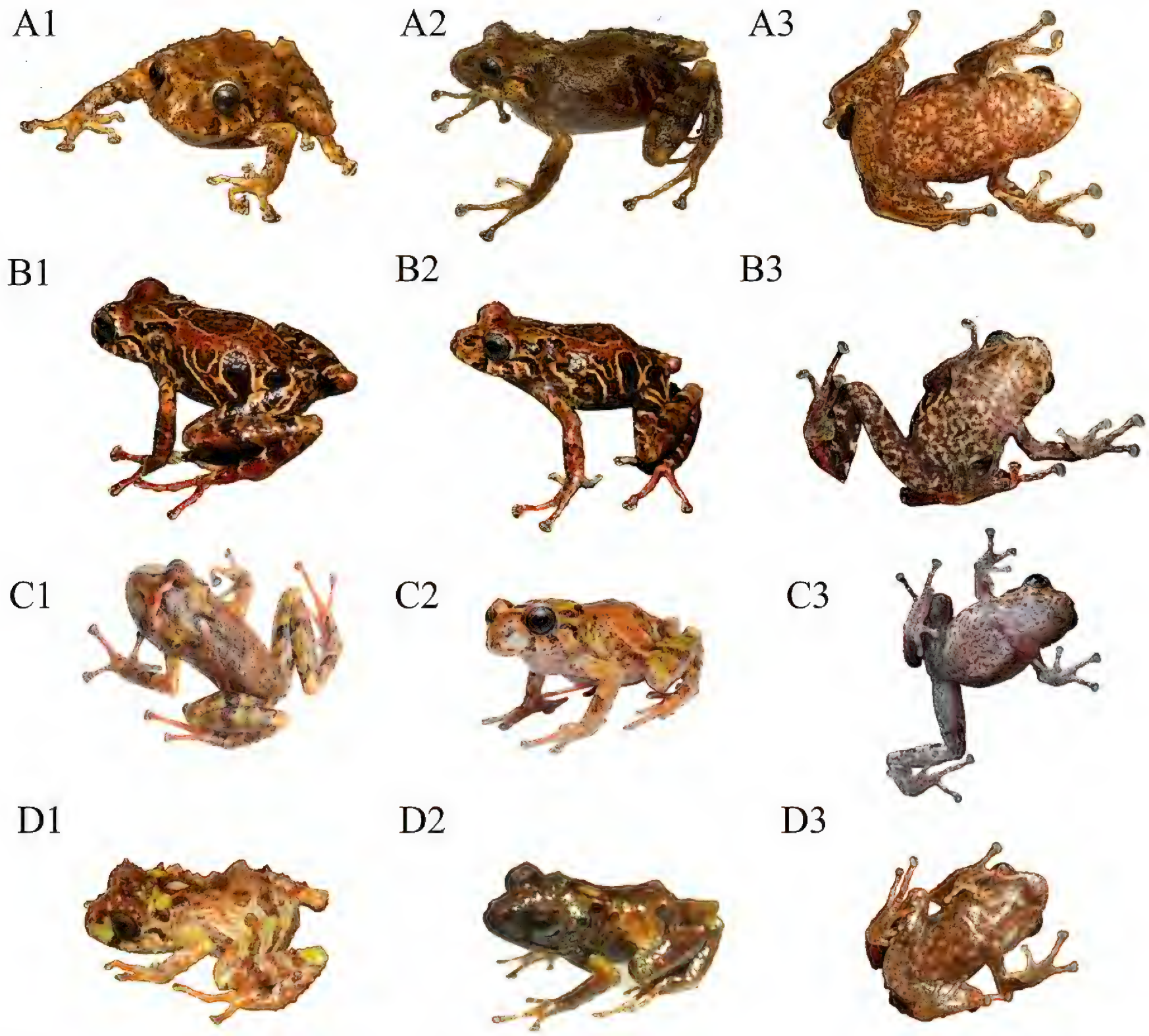

Figure 5. Life coloration variation of the type series of *Pristimantis normaewingae* sp. nov. **A1–A3.** DHMECN 18863, adult female; **B1–B3.** DHMECN 18404, adult male; **C1–C3.** DHMECN 17191, juvenile; **D1–D3.** DHMECN 18996 male juvenile. Photographs JPRP and ZL.

Variation. (Figs 5, 6) Morphometric variations are shown in Table 3. In preservative, the new species presents a wide gradient of banded patterns in dorsal and limb coloration, with backgrounds ranging from pale cream tones, different dark brown tones, and even greenish with orange tones; dark marks on the flanks are delineated by thin pale lines. Ventrally specimens vary with different tones of light brown peppered with gray flecks, especially on the throat, heavily marmorated with dark brown irregular marbling; some specimens present white dots irregularly dispersed on the body.

In life, polychromatic, sexual dimorphism. In dorsal view, females dark brown to pale brown with scapular and sacral marks less conspicuous than males. Iris coloration pale gray with a medial horizontal line reddish brown finely dark reticulated, white sclera (DHMECN 18409, DHMECN 18863). Males with distinctive dorsal and flank coloration ranging from reddish brown to orange brown. P osterior surface of head and scapular region ranging from light brown to yellowish green l ines surrounding marks on the of flanks ranging from white to pale ochre. Sacral marks ranging from reddish, orange, and dark brown. Iris variation in males with blueish gray with a coppery (DHMECN 18400, 18392) or faint reddish brown medial horizontal line (DHMECN 18404). Ventrally, males and females with a grayish brown background with dark brown blotches, yellow cream, or grayish cream. Juveniles with pale or dark and light brown, interorbital region dark brown (DHMECN 18996) or with interorbital line orange brown (DHMECN 17191).

In life, *Pristimantis normaewingae* sp. nov. shows some variation in tuberculation. Males show eyelids, interorbital and scapular tubercles elevated and conical (DHMECN 18400, 18392, 18996). Females only have small, rounded, and conical tubercles (DHMECN 18409, DHMECN 18863). Some individuals show plasticity in the state of tubercles texture under different environmental conditions, ranging from elevated spiny to relaxed low tuberculated state, additionally preservation could reduce protuberance of tubercles.

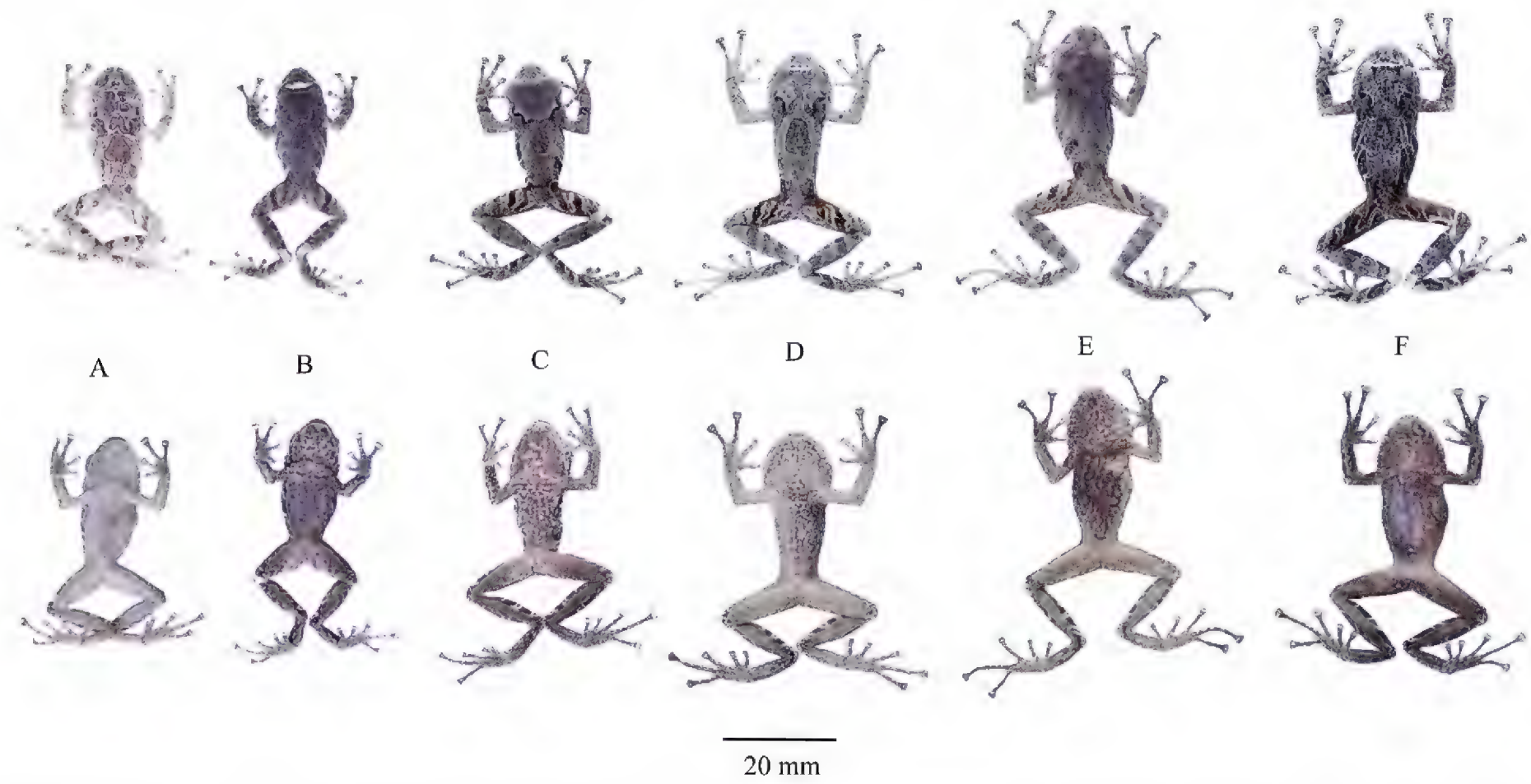

Figure 6. Color pattern variation in preserved specimens of *Pristimantis normaewingae* sp. nov. **A.** DHMECN 18400, male; **B.** DHMECN 18393, male; **C.** DHMECN 18222, male; **D.** DHMECN 18223, female; **E.** DHMECN 18228, male; **F.** DHMECN 18212, male. Photographs JPRP.

Etymology. The specific epithet *normaewingae* is a noun in the genitive case and is a patronym in honor of Norma Ewing, a passionate conservationist and animal rescue volunteer from United States. Her belief that all species, and especially frogs, are interconnected, is rooted in her early childhood surrounded by nature living and working on a farm. She has always cared for animals of all shapes and sizes. As a volunteer with animal rescues, she was admired for her ability to nurture orphaned and injured animals.

Distribution, natural history observations, and conservation status. (Figs 7, 8) *Pristimantis normaewingae* sp. nov. is known only from the type locality in the Cerro Candelaria Protected Area at an elevation of 2550 to 3150 m. The collection site is eastern Andean cloud forest with a dominance of bromeliads in the forest floor and in the branches of the trees and shrubs, *Clusia* trees, palms, and *Weinmannia*, with a high density of epiphytes, mosses, ferns, lichens, and orchids. Sympatric species found with the new species were *Pristimantis gladiator complex*, *P. tungurahua*, *P. marcoreyesi*, and *Niceforonia* sp.

Because of the lack of records, evidence of population status, and geographic distribution, we recommend assigning *Pristimantis normaewingae* sp. nov. to the Data Deficient IUCN Red List Category (based on IUCN, 2019 guidelines), however, the only known population is protected within Cerro Candelaria Private Protected Area.

New records of *Pristimantis maryanneae* Reyes Puig et al., 2018

For the first time, sequences of the holotype (DHMECN 14454) of *Pristimantis maryanneae* are integrated into a phylogenetic analysis (Fig. 1), and are concordant with those of new specimens from surrounding mountains in the upper Pastaza valley like Finca Palmonte (DHMECN 18212, 18213, 18215, 18216, 18217, 18218, 18220, 18221, 18224, 18225, 18227), and Bosque Protector Hacienda Guamag (DHMECN 18228, 18222, 18227, 18224, 18221, 18225, 18226) (Fig. 7). These results validate our morphological descriptions (Reyes-Puig et al. 2022) and corroborate the monophyly of the species.

With the inclusion of new populations, more coloration morphs are evident (Figs 4, 9) for this species. Those coloration patterns range from white pale cream to orange, yellow, and light brown, present irregular dark marks, or longitudinal striped patterns, even a pale pattern without distinctive marks. Some juveniles have dark or green colorations, and oval marks on the sacrum are complete. Ventral coloration is predominantly light grey, light brown, or white with minute black peppering, and always presents a mid-ventral thin dark line, and some individuals have faint yellow or pink tones on shanks. Measurements of *Pristimantis maryanneae* are presented in Table 3.

Pristimantis maryanneae is only known from four localities, two north and two south to the upper Pastaza valley (Fig. 8). All localities are in the upper montane forest 2300–2600 m under conservation strategies by local NGO Ecominga, Finca Palmonte and Bosque Protector Hacienda Guamag owners. The species inhabits cloud forest with a dominance of ferns on the forest floor, abundant epiphytic vegetation including bromeliads, orchids and lichens in the floor and the branches of the trees and shrubs, along with *Clusia* trees and *Ceroxylum* palms.

Sympatric species found with *Pristimantis maryanneae* on the south side of the Rio Pastaza in Guamag were

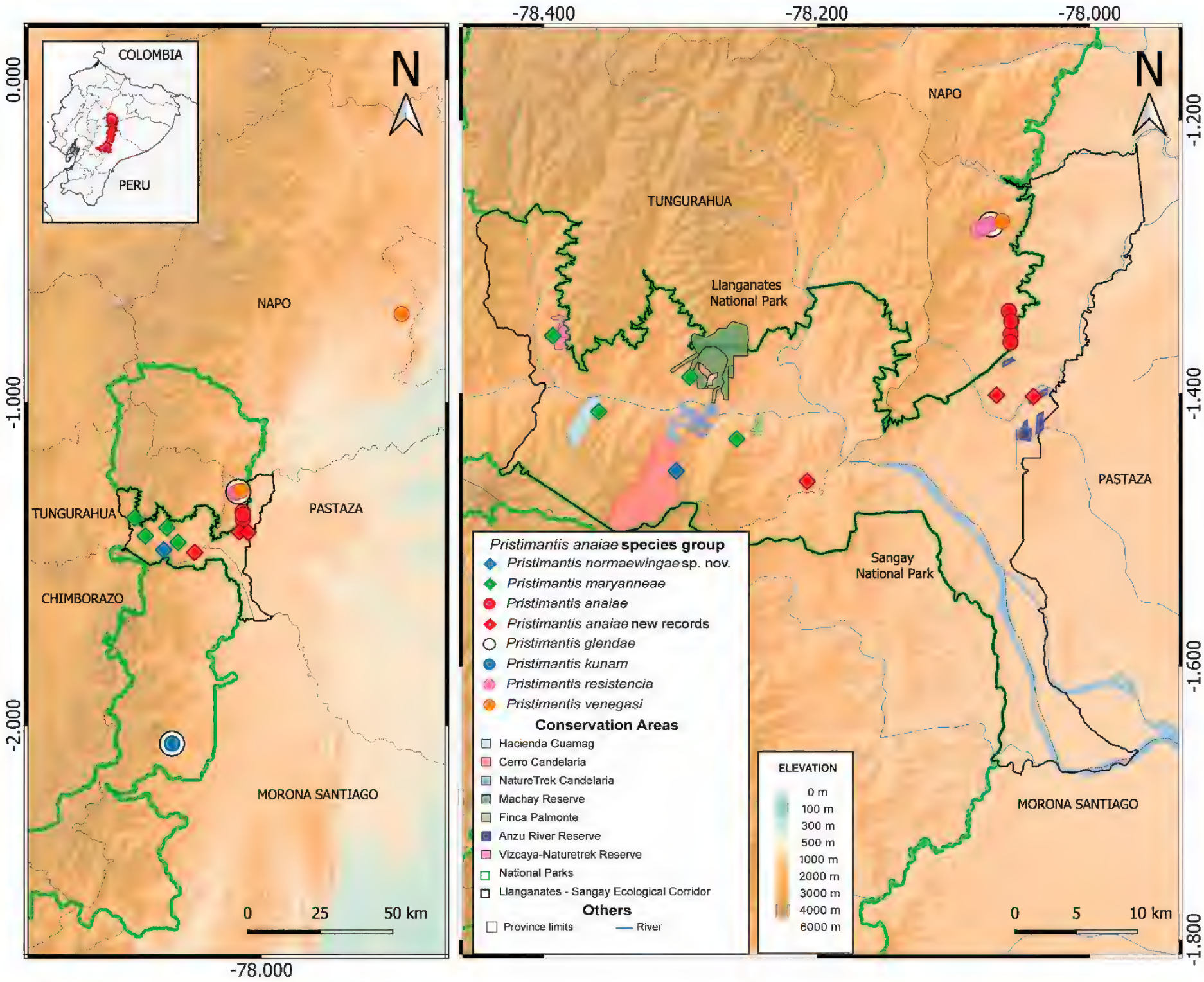

Figure 7. Distribution map of the *Pristimantis anaiae* species group including a new species from the mountains south of the Pastaza River.

P. donnelsoni, *P. marcoreyesi*, *P. tungurahua*, *P. yanezi* and *Noblella naturetreki*; whereas in Finca Palmonte (also on the south side of the Rio Pastaza) it occurs with *P. marcoreyesi*, *P. eriphus*, *Osornophryne* cf. *simpsoni* and *Caecilia abitahuae*. In Cerro Mayordomo and Vizcaya Naturetrek Reserve on the north side of the Rio Pastaza the species was sympatric with *P. marcoreyesi* and *P. kayi*.

Because of the lack of records, evidence of population status, and geographic distribution, we recommend assigning *Pristimantis maryanneae* to the Data Deficient IUCN Red List Category (based on IUCN, 2019 guidelines).

Discussion

Our new phylogeny added four species and updates the results and data presented by Ortega et al. (2022). The *Pristimantis anaiae* species group contains two candidate species and following seven species: *Pristimantis anaiae* (Ortega et al. 2022), *P. glendae* (Ortega et al. 2022), *P. maryanneae* (Reyes Puig et al. 2022), *P. kunam* (Ortega et al. 2022), *P. resistencia* (Ortega et al. 2022), *P. venegasi* (Ortega et al. 2022), and *P. normaewingae* sp. nov.

Pristimantis kunam was the most basal lineage followed by *P. glendae* as the second most basal lineage based on the phylogenetic inference hypothesis made by Ortega et al. (2022). Our topology differs from Ortega et al. (2022) and shows two well-supported subclades within the group. With the inclusion of *P. normaewingae* sp. nov. and *P. maryanneae*, evolutionary history within the clade had changed. As new lineages were included new topologies and clades had been formed. However, *Pristimantis anaiae* species group maintains its strong support (100/100) like the phylogeny from Ortega et al. (2022). Also, support values in the clade *P. venegasi* + *P. anaiae* (68.6/53 branch support) presented in the phylogeny by Ortega et al. (2022) are increased in our phylogeny, *P. venegasi* + (*P. anaiae* + *P.* sp. 1) (85.8/99 branch support).

Morphologically, the presence of large sacral dark round areas with thin clear borders was reported by Ortega et al. (2022) (Fig. 4) as a putative synapomorphy of the *Pristimantis anaiae* species group, same as suggestion made Bejarano-Muñoz et al. (2022), we consider a comprehensive analysis of the distribution of these coloration

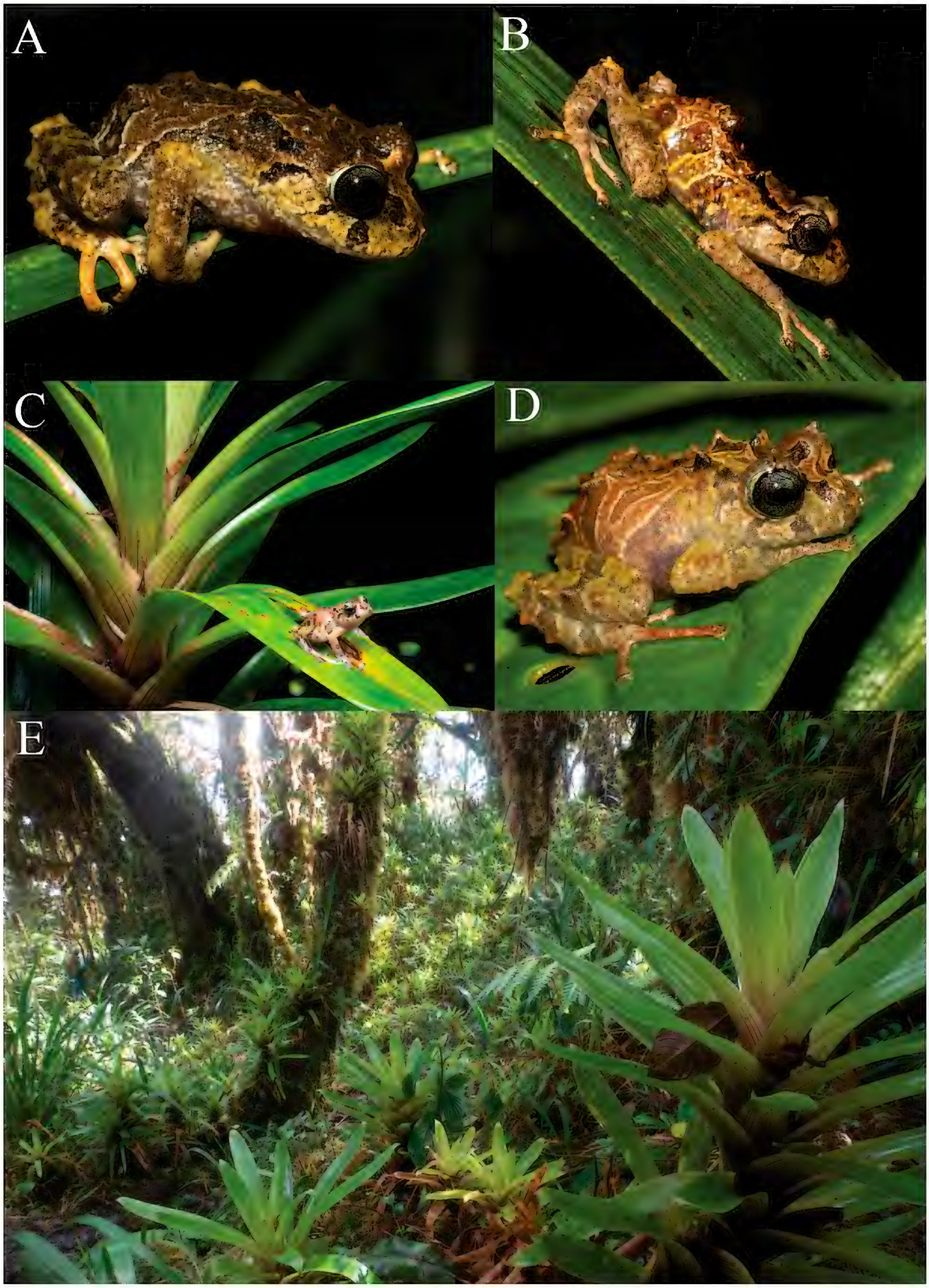

Figure 8. *Pristimantis normaewingae* sp. nov. in its habitat at Cerro Candelaria. **A.** DHMECN 18868, female; **B.** DHMECN 18401, male on *Neurolepis aristata* (Poaceae) at night; **C.** DHMECN 17188, juvenile on bromeliad at night.; **D.** DHMECN 18400, male on leave of shrub; **E.** habitat at the type locality of the new species in Cerro Candelaria. Photographs JPRP and ZL.

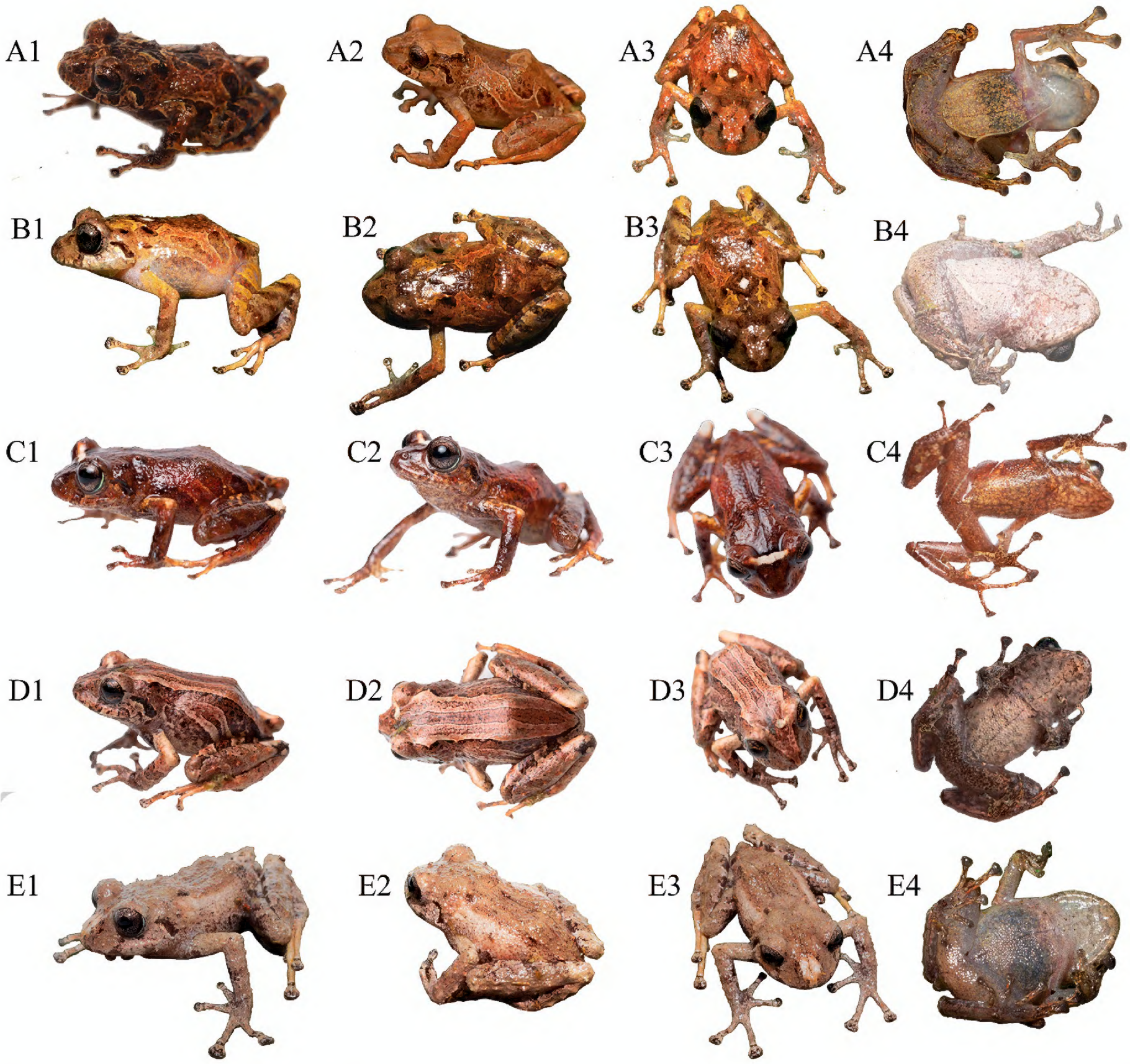

Figure 9. Life coloration variation of *Pristimantis maryanneae*. **A1.** DHMECN 14452, paratype adult male from Naturetrek Vizcaya Reserve; **A2.** DHMECN 19093, adult female from Machay Reserve, Cerro Mayordomo; **A3, A4.** DHMECN 18225, adult male from Bosque Protector Hacienda Guamag; **B1–B4.** DHMECN 18227, adult male from Bosque Protector Hacienda Guamag; **C1–C4.** DHMECN 18216, adult male from Finca Palmonte; **E1–E4.** DHMECN 18223, adult female from Bosque Protector Hacienda Guamag. Photographs JPRP, JCC, MYM, and PV.

patterns is needed to determine if these characters are homologous or homoplastic, sacral marks are present in other species of *Pristimantis* from distant species groups (e.g. *P. katoptroides*, *P. verecundus*).

Geographical distribution of the *Pristimantis anaiae* species group encompasses eastern Andean slopes of central Ecuador, from Sumaco volcano at its northern limit to the central part of Sangay national park at its southern limit. Until now, our data suggest that the upper Rio Pastaza watershed is the center of adaptive radiation, were most species of the group are concentrated (*P. anaiae*, *P. glendae*, *P. resistencia*, *P.venegasi*, *P. normaewingae*, plus two undescribed species included in our phylogeny); at both northern and southern extreme latitude distributions of the group, only one species occurs (Fig. 7).

Pristimantis anaiae species group members have been found between 1200 and 3150 m on low vegetation and medium-sized bushes, ferns, and Poaceae, in cloud forest and high Andean Forest. *P. venegasi* on the north, plus *P. glendae* and *P. kunam* on the south are the peripheral species of the group, whereas the mountains immediately to the north (Llanganates) and south (Sangay) of the Rio Pastaza valley hold the highest diversity and endemism of the group, from 2000 to 3000 m, suggesting that like in another groups (e.g., Lynch and Duellman 1980), the Rio Pastaza and surrounding mountains, become a geologic ancient area of explosive radiation, with speciation-diversification as a result of the microclimatic variation of the ecosystems from each mountain to another (Jost 2004).

New collections in the foothills of the southern and northern Rio Pastaza watershed reveal additional localities for poorly known species in the *Pristimantis anaiae* species group (Fig. 7), which mean that additional samples from surrounding mountains in the upper Rio Pastaza watershed (intermediate altitudes of Cerro Negro and Cerro Candelaria eastern slopes, Llanganates mountains), would help in the understanding of the evolution of the *Pristimantis anaiae* species group in these mountains.

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Appendix 1

Specimens examined:

Pristimantis venegasi: Napo: Parque Nacional Sumaco Galeras MZUTI 6571, MZUTI 6574, MZUTI 6575.

Pristimantis normaewingae sp. nov.: Tungurahua: Cerro Candelaria Private Protected Area, DHMENC 18863, DHMECN 18861, DHMECN 18865, DHMECN 18868, DHMECN 4849, DHMECN 4846, DHMECN 18409, DHMECN 18996, DHMECN 18860, DHMECN 18397, DHMECN 18411, DHMECN 18404, DHMECN 18403, DHMECN 18399, DHMECN17190, DHMECN 18866, DHMECN 18393, DHMECN 18401, DHMECN 18392, DHMECN 18414, DHMECN 18396, DHMECN 18400, DHMECN 18896, DHMECN 18862, DHMECN 18402, DHMECN 18412, DHMECN 18407, DHMECN 18406, DHMECN 18408, DHMECN 17189, DHMECN 17191, DHMECN 17188, DHMECN

17192, DHMECN 4847, DHMECN 4975, DHMECN 18405, DHMECN 18413, DHMECN 18398, DHMECN 18400, DHMECN 18864.

Pristimantis maryanneae: Tungurahua: Machay Reserve, Cerro Mayordomo: DHMECN 19093. Finca Palmonte, DHMECN 18223, DHMECN 18228, DHMECN 18222, DHMECN 18215, DHMECN 18217, DHMECN 18227, DHMECN 18213, DHMECN 18224, DHMECN 18218, DHMECN 18216, DHMECN 18221, DHMECN 18220, DHMECN 18212, DHMECN 18225, DHMECN 18219, DHMECN 18214, DHMECN 18226, DHMECN 14454, DHMECN 14451, DHMECN 14452, DHMECN 14550

Pristimantis anaiae: Pastaza, Parque Nacional Llanganates, Comunidad Zarentza, QCAZ 59640, Sumak Kawsay In situ DHMECN 16859 DHMECN 16860; Tungurahua: Finca El Encanto, propiedad de Diego Muñoz, DHMECN 16249, DHMECN 16250.